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BIOLOGICAL APPLICATIONS AND EFFECTS OF OPTICAL MASERS. (U)

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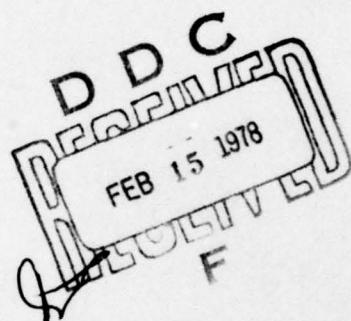
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BIOLOGICAL APPLICATIONS AND EFFECTS OF OPTICAL MASERS

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RESEARCH PROGRESS



Report No. 677V

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1. Photopathology of Retinal Lesions Induced by Short Wavelength Light

Our most significant contribution during the past year has been the histological and morphological characterization of the photochemical lesion resulting from the exposure of the rhesus retina to blue light (441.6 and 435 nm).

Retinal lesions on the rhesus fundus were produced with the 2500 W xenon lamp in conjunction with narrow bandpass filters at 441.6 and 435.8 nm rather than with laser sources because the latter produce a Gaussian distribution of irradiation on the retina, whereas the xenon source has almost uniform irradiance across the retinal spot. For the purpose of histological study, most of these lesions were somewhat above threshold (30 mW/cm² on retina for 1000 s) and 1 mm in diameter. Exposures were for 1000 s in all cases, except for two eyes which were exposed for 10,000 s. Threshold response is taken to be the appearance of a funduscopically visible lesion at two days postexposure. No lesions are visible, even for exposures twice threshold level, until 48 hours postexposure. The photopathology in what follows explains why this latency in the appearance of the lesion occurs. Reciprocity seems to hold for exposure times of 1000 and 10,000 s, the radiant exposure being about 30 J/cm² for both exposure durations.

After an eye is enucleated, it is transected at the base of the cornea and flaps are cut to open up the eye. It is immersed in fixative (10% acrolein in 0.1 M cacodylate buffer) at room temperature and allowed to stand for 30 minutes. Then, working in a fume hood using a stereomicroscope and a polyethelene dish containing a Teflon disc as a working surface, the flaps are trimmed away and the samples (funduscopically visible lesions 1 mm in diamter) are removed with a 2 mm trephine. The tissue plugs are transferred to BEEM capsules containing 0.1 M cacodylate for rinsing. The buffer is pipetted off and fixative (2% OsO₄ in 0.1 M cacodylate buffer) is added. The specimens are postfixed for 1 hour at room temperature. The specimens are rinsed with 0.1 cacodylate and then dehydrated in an acetone series. The samples are stained in block during dehydration by adding 2% uranyl magnesium acetate to the various lower concentrations of the acetone series. Then the specimens are infiltrated with low viscosity plastic, transferred to conical BEEM capsules with flattened tips, oriented for optimal sectioning position, and placed in an oven to polymerize at 70°C for about 24 hours.

Sectioning is done on a Sorvall MT-2B ultramicrotome. During facing of the block, the tissue plug is oriented so that the plane of section is perpendicular to the layers of the retina. The lesion is localized by light microscopy of serial thick sections of the sample. The thick sections (2.5-3 µm) are cut on a dry glass knife, transferred to small drops of water oriented in series on a clean slide, and dried on a hot plate (60-70°C). The dried sections (10 per slide) are stained by covering them with 0.5% toluidine blue in 1% sodium borate and heating them on the hot plate for 20-40 seconds. The stain is gently rinsed off with distilled water, the slide dried under a hot air dryer and the sections mounted under a cover slip using Permount. When the lesion is located (maximum length is 1 mm within a 2mm sample plug), it is bounded by normal tissue, providing an internal control for the interpretation of histopathology.

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After a region of the block is chosen for study by electron microscopy, the block face is trimmed down and thin sections (gold and silver interference colors) are cut, using a Ladd diamond knife. The sections are mounted on 300 mesh copper grids, poststained with lead citrate (or uranyl acetate followed by lead citrate), and viewed in an electron microscope.

Serial thick sections are studied by light microscopy using a Bausch & Lomb research microscope. Micrographs are obtained on a camera-equipped Zeiss microscope to which access is generously provided by Dr. R. Geeraets of the Department of Ophthalmology.

Thin sectioned material is studied and photographed on an Hitachi HU-12 high resolution transmission electron microscope (a multi-user facility of the School of Basic Sciences).

In the near future the School of Basic Sciences will obtain a high resolution scanning electron microscope equipped with an X-ray elemental microanalyzer. When this tool becomes available, we will use it judiciously in our research effort.

Over 3500 sections on 20 eyes have been processed at times ranging from one hour postexposure to 90 days. The results up to 30 days postexposure are summarized in Tables 1, 2, 3 and 4. More recent data on 60 and 90 days postexposure will be discussed below. Most of these data are confined to the paramacular area of the rhesus retina. The yellow macular pigment which is very prominent in the rhesus retina protects the fovea by absorbing strongly in the blue region of the spectrum. It requires roughly twice the threshold irradiance in the paramacular area to produce a lesion in the fovea.

For specimens taken from eyes enucleated within one hour postexposure, the neural retina, retinal pigment epithelium (RPE) and the choroid appear essentially normal (Table 1). In the case where 46 mW/cm^2 impinged on the retina for 1000 s (this is well above threshold) a few pyknotic rod nuclei and some darkly stained cone inner segments were noted at one hour postexposure. These same observations held for an exposure to 33 mW/cm^2 at one day postexposure. The neural retina, RPE and choroid still appeared essentially normal. We do detect some very limited damage to the photoreceptors immediately and one day postexposure which may help to explain the results of Sperling and Lawwill (Science 174, 520, 1971; Inv. Ophthal. 12, 45, 1973).

At 48 hours post-exposure the picture changes dramatically (see Tables 1 and 3). We now observe definitive inflammation of the RPE accompanied usually but not always by inflammation of the choroid. At this time there are no detectable changes in the photoreceptors other than those noted above at one hour and one day postexposure. We have chosen to denote this as pigmentary retinochoroiditis. It is a typical inflammatory response to insult. The melanosomes in the apical space of the RPE have been aggregated and engulfed or phagocytized by macrophages, presumably sent in by the reticuloendothelial system, though there is the possibility that RPE cells have differentiated into macrophages. This is unlikely because quite frequently at 48 hours postexposure the choriocapillaris is engorged with white cells. It is the aggregation of the melanosomes by the macrophages, producing hypopigmentation of the RPE, which makes the lesion funduscopically visible at 48 hours postexposure. These macrophages with their phagocytized melanin debris are situated primarily in the sub-retinal space where they remain for at least 30 days. This train of events seems to indicate that the melanosomes or melanin granules are the initial site of injury.

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Table 1

Paramacular response to short wavelength light (441.6 nm) after irradiation at a 1 mm spot for 1000 seconds. Threshold irradiance is approximately 30 mW/cm² at this wavelength and duration of exposure.

<u>age</u>	<u>irradiance at retina</u>	<u>description of response</u>
1 hour	33 mW/cm ²	appears essentially normal
1 hour	*46 mW/cm ²	some pyknotic nuclei in outer nuclear layer (rods); some dark-stained cones
1 day	33 mW/cm ²	a few pyknotic nuclei in outer nuclear layer; some dark-stained cones
2 days	33 mW/cm ²	pigmentary retinochoroiditis: macrophages in subretinal space
2 days	35 mW/cm ²	pigment epitheliitis: macrophages in subretinal space
3 days	35 mW/cm ²	pigmentary retinochoroiditis: some dark-stained cones; melanophages in subretinal space; edema in choroid
5 days	35 mW/cm ²	pigment epitheliitis: depigmented PE cells; melanophages in subretinal space
6 days	33 mW/cm ²	pigmentary retinochoroiditis: mitoses in choroid; depigmented PE cells; melanophages in subretinal space; outer segment degeneration
6 days	35 mW/cm ²	pigment epitheliitis: mitoses at level of PE, (reactionary hyperplasia); melanophages in subretinal space; outer segment degeneration
10 days	35 mW/cm ²	pigment epitheliitis: depigmented PE cells; melanophages in subretinal space; outer segments normal
11 days	35 mW/cm ²	pigment epitheliitis: depigmented PE cells; melanophages in subretinal space; outer segments normal
30 days	35 mW/cm ²	regenerated pigment epithelium hypopigmented; melanophages in subretinal space; outer segments fully regenerated

*spot size was 1.4 mm

Table 2

Paramacular response to short wavelength light (435.8 nm) after irradiation at a 1 mm spot for 1000 seconds. Threshold irradiance is approximately 20 mW/cm^2 at this wavelength and duration of exposure.

<u>age</u>	<u>irradiance at retina</u>	<u>description of response</u>
5 days	35 mW/cm^2	pigment epitheliitis: depigmented PE cells; melanophages in subretinal space; outer segment degeneration
11 days	35 mW/cm^2	pigment epitheliitis: depigmented PE cells; melanophages in subretinal space; some pyknotic nuclei in outer nuclear layer (rods & cones); dark chromatin clumps in nuclei at level of horizontal cells; some dark-stained cones; outer segment degeneration; dark-stained Muller cell
30 days	35 mW/cm^2	regenerated pigment epithelium hypo-pigmented; melanophages in subretinal space; some dark-stained cones; regeneration of outer segments

Table 3

Paramacular response to short wavelength light (441.6 nm) after irradiation at a 1.4 mm spot for 10,000 seconds (2.8 hours). Threshold irradiance is approximately 3 mW/cm^2 at this wavelength and duration of exposure.

<u>age</u>	<u>irradiance at retina</u>	<u>description of response</u>
2 days	5 mW/cm^2	pigmentary retinochoroiditis: some dark-stained cones; macrophages in subretinal space

Table 4

Macular response to short wavelength light (441.6 nm) after irradiation at a 1.4 mm spot for 10,000 seconds (2.8 hours). Threshold irradiance for paramacular response is approximately 3 mW/cm^2 at this wavelength and duration of exposure.

<u>age</u>	<u>irradiance at retina</u>	<u>description of response</u>
30 days	10 mW/cm^2	pigment epitheliitis: hyperplasia of PE; depigmented PE cells; outer segment degeneration; melanophages in subretinal space
30 days	18 mW/cm^2	hypopigmented PE; outer segments partly regenerated; melanophages in subretinal space; edema in choroid

Otherwise, it is difficult to explain why the macrophages take up the melanosomes. The photoreceptor cells appear essentially normal at 48 hours post exposure and also at 72 hours postexposure.

Five and six days after exposure (Tables 1 and 2) light microscopy reveals additional details about the inflammatory response to photic insult. The RPE shows reactionary hyperplasia with RPE cells juxtapositioned on top of each other and relatively devoid of melanin. Mitotic figures are found in both the RPE and choroid. The outer segments (OS) of the photoreceptor cells are disorganized and show degeneration. In some cases the choroid shows marked inflammation with the choriocapillaris filled with white cells and very few erythrocytes are present. In other cases the choroid looks normal but there is always inflammation and hyperplasia in the RPE and degeneration of the OS of the photoreceptors. This evidence suggests again that the initial site of damage is in the RPE. OS degeneration does not set in until later when the metabolic supportive role of the RPE is endangered. This demonstrates that the metabolic maintenance of the photoreceptors is dependent on the well-being of the RPE. It does not exclude, of course, the possibility of photo-oxidative damage to the plasma and disk membranes of the OS. Photic insult to these membranes could occur simultaneously with that to the RPE. Such damage would then be accentuated when the RPE becomes inflamed. While phagosomes in the RPE have the ability to digest OS debris, there is no evidence in any of our observations that the macrophages present in the subretinal space can digest the melanin which they have phagocytized. Quite frequently one can observe both OS debris and melanin in the same macrophage.

Observations at 10 and 11 days postexposure (Table 1 and 2) show continued but much reduced pigment epitheliitis with hypopigmentation of the RPE and macrophages filled with melanosomal debris in the subretinal space. Hyperplasia in the RPE is no longer seen and the cells are well lined up in cuboidal array but relatively devoid of pigmentation. The outer segments of the photoreceptors have regenerated and look almost normal except that they are somewhat shorter than those in the surrounding unirradiated tissue.

At 30 days postexposure the RPE has regenerated completely and looks normal except that there are relatively few melanin granules in these cells while macrophages containing melanin debris are still located in the subretinal space. There is no indication that this melanin has been digested. Apparently there is no enzyme system in the body which can digest this material either in skin or ocular tissue. The photoreceptors and the choroid look normal at 30 days but the site of the former lesion is still visible funduscopically because of the hypopigmentation of the RPE. Very recent observations at 60 and 90 days show that the macrophages in the subretinal space have disappeared, leaving the RPE hypopigmented. The site of the lesion is still funduscopically visible, though faintly. There is no way of knowing how these macrophages left the subretinal space but we assume they left via Bruch's membrane. Some support for this route of exit is the observation that during the later stages of the lesion recovery some macrophages were found at the base of the RPE next to Bruch's membrane.

Figure 1 is a schematic diagram in which we have devised a general scheme of retinal photopathology which illustrates the train of events we have observed and contrasts them with similar events in the albino rat syndrome. The pathway of events illustrated by the thick solid arrows on the left side of the diagram depicts retinal response after long term exposures to short wavelength light at low levels of irradiance as we have observed them. The thin solid arrows on the right side of the

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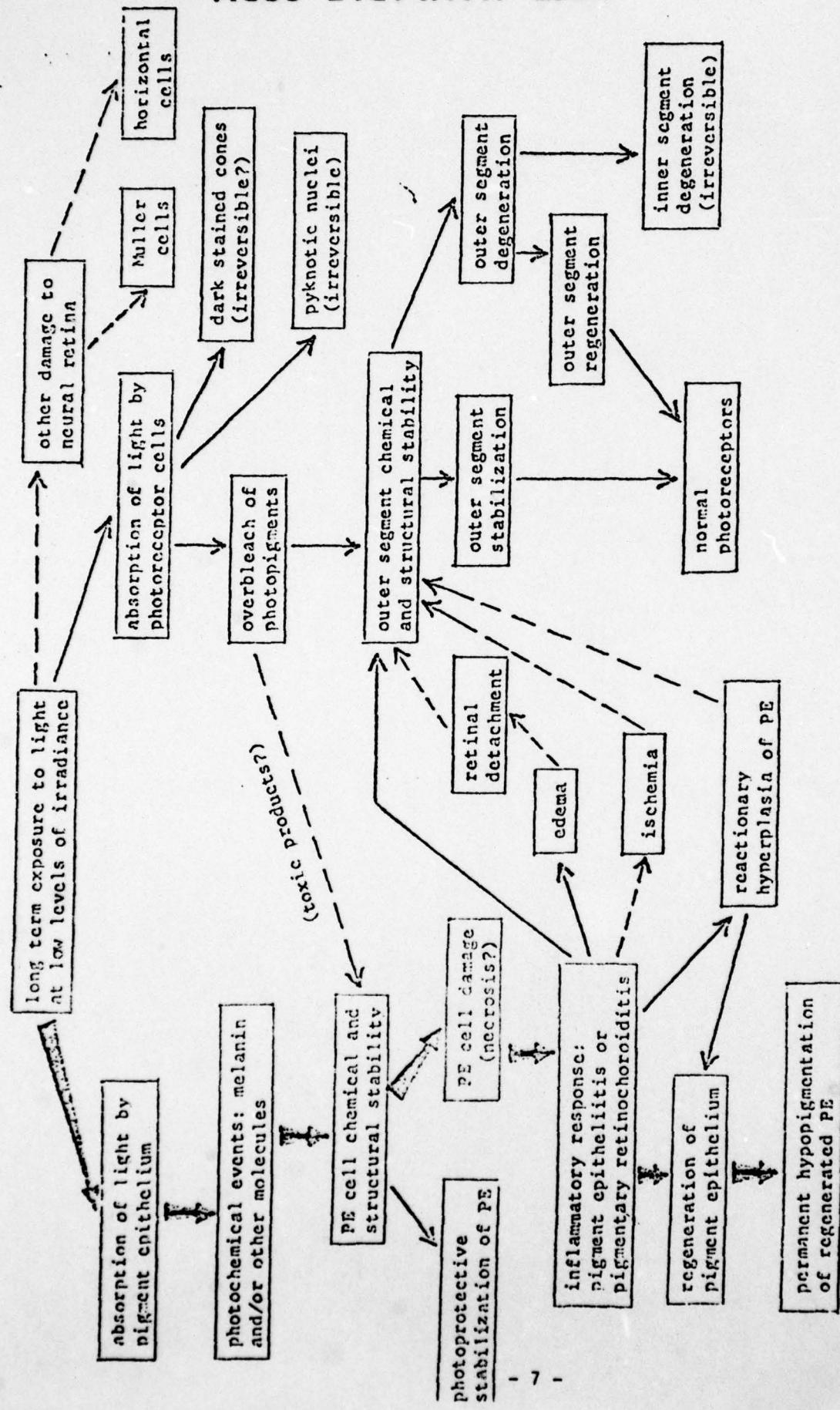


Figure 1

GENERAL SCHEME OF RETINAL PHOTOPATHOLOGY: pathways of retinal response after long term exposures to short wavelength light at low levels of irradiance. Histopathology of the threshold level lesion (funduscopically visible lesion 48 hours after treatment) is characterized by pathway of thick solid arrows. Thin solid arrows indicate both alternative paths for subthreshold exposures and additional pathways of photopathology. Dashed arrows indicate accessory pathological pathways that can occur.

diagram show the course of events in the albino rat syndrome. These thin solid lines indicate both alternative pathways for subthreshold exposures and additional pathways of possible photopathology. The dashed arrows suggest still other pathways which might interrelate and accentuate the train of events.

2. Relationship Between Retinal Lesions Produced by Short Wavelength Light and Visual Performance in the Trained Rhesus Monkey

Although our Army Contract did not finance the training program for visual performance in the rhesus monkey, we report the results here because of their vital significance in assessing the lesion produced by short wavelength light.

Very briefly the animals are trained to sit in a restraint chair inside a sound-proof chamber and press one of two available levers upon the appearance of a Landolt C on a screen in front of the chair. The gap in the Landolt C subtends an angle at the animal's eye which corresponds to 20/10, 20/20, 20/30, etc. vision, similar to a Snellen chart. A spatial relationship exists between the gap orientation and the correct response lever, the right lever corresponding to the gap on the right side of the C and the left lever to the gap on the left side. Subjects trained to perform this discriminatory task are rewarded with a 300 mg banana-flavored food pellet for each correct response. Animals are trained monocularly on a daily basis, each training session lasting about 40 minutes and consisting of 96 trials per eye. A patch over the unused eye insures monocular vision. A trial consisted of the appearance of the stimulus and its subsequent removal by the animal's response or failure to respond within 5 s. An 8 s inter-trial period followed, during which time the levers were deactivated. Failure to respond within 5 s was considered incorrect and went unrewarded.

Animals, on the average, require about 3-4 months to be trained to perform this visual task. Thereafter, they get approximately 90% of the 20/20 task correct, and nearly 100% for 20/30. Their performance at 20/10 is spotty and rarely significant. Statistical analysis predicts that 69% correct is significant to the P 0.01 level. That is, there is only one chance in a hundred of getting 69% correct by chance in 32 trials.

Trained animals have been exposed for 1000 s to blue light (441.6 nm) at one, two and three times the threshold level as determined in the paramacular area. These exposures were performed with the 2500 W xenon lamp using an interference filter with 6 nm bandwidth at the half power levels. The spot size on the retina was 1 mm in diameter and centered on the fovea centralis. Figure 2 is a plot of percentage correct vs time in days for an animal whose left eye was exposed to threshold level (30 mW/cm^2 on retina) for 1000 s. The upper set of lines gives 20/30, 20/20, and 20/10 both before and after exposure while the lower set of lines shows similar performance in the control (unexposed) eye. Straight lines represent the 69% significance level. At one day postexposure there is a significant drop in 20/20 vision for both the exposed and the control eye. We attribute this to the deep anesthesia which the animal received on the day of exposure. This anesthesia effect has appeared in 3 trained animals for the first two or three days following exposure. Otherwise the exposed eye returned to 20/20 vision after the third day, though not as rapidly as the control eye. We conclude that a paramacular threshold exposure in the macular area does not significantly impair vision in the rhesus monkey.

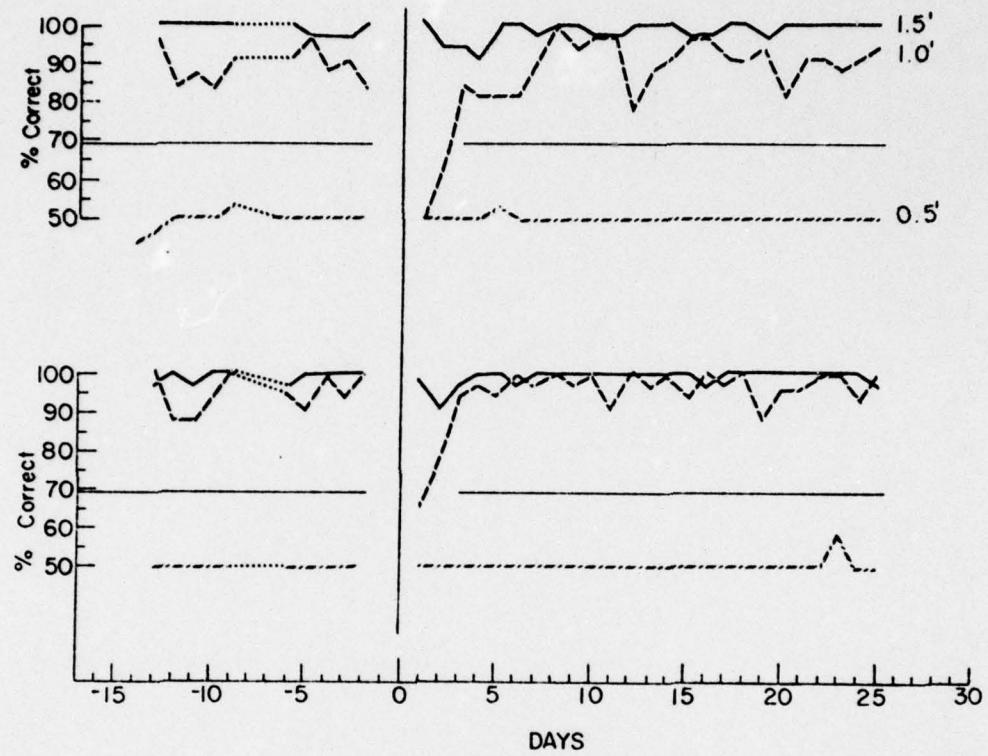


Figure 2

1 x Threshold (30 mW/cm² for 1000 s)

20/10 = 0.5°

20/20 = 1.0°

20/30 = 1.5°

Figure 3 illustrates the effects on vision of an exposure which was twice paramacular threshold. This time the right eye in the same animal was exposed while the left eye previously exposed to threshold served as control. The anesthesia effect is apparent for both eyes, but the exposed eye shows a very significant loss in 20/20 vision for a period of 20 days. Even 20/30 vision drops below the 69% line on day 7 postexposure. After the 20th day, 20/20 vision gradually returns to normal at about the 29th day. Thereafter, the exposed eye displayed no loss in visual performance; in fact, we used this eye as the control eye in the next experiment.

In Figure 4, the effects on visual function are shown for an exposure of thrice threshold, still in the same animal, whose left eye is exposed while the right eye, previously exposed to twice threshold, serves as control. There is a definite loss of 20/20 vision in this eye which is permanent up to 84 days postexposure (the latest data on this animal).

We have performed similar experiments on two other animals and conclude the following:

- (a) The paramacular area is more sensitive than the macular area by almost a factor of two. Paramacular threshold exposure to the macula (30 mW/cm^2 for 1000 s) does not impair visual acuity in the rhesus monkey. Twice paramacular threshold does impair vision, but the ability to perform at 20/20 returns to normal in about 30 days. Three times paramacular exposure to the macula produces permanent impairment (90 days).
- (b) The loss and eventual recovery of visual function for exposures up to twice paramacular threshold parallels the photopathology discussed previously in Section 1. That is, loss of visual function occurs about the sixth or seventh day postexposure when the outer segments first show disorganization and degeneration. Between 10 and 30 days postexposure the outer segments of the photoreceptors undergo regeneration with return of 20/20 visual performance. Judging by Figure 3, this recovery begins about the 19th day postexposure and is completed by the 30th day.
- (c) The yellow macular pigment plays an important role in protecting the macular area from short wavelength light.

3. Current Status of 2500 W Xenon Lamp with Quartz Optics

Construction of this optical source was completed last October. This source represents a considerable improvement over the earlier xenon source in power, flexibility and ability to produce both infrared and ultraviolet radiation. Figure 5 is a schematic diagram of the apparatus. The quartz collector lens system is placed much closer to the xenon lamp than the collecting system in the old source. This increases the power output by a considerable factor. With no filters in the system and a 9.5° diverging beam the output is 5.84 Watts.

The focussed image of the lamp is 8 mm in diameter. By varying the size of this image with the image diaphragm in conjunction with a quartz lens of suitable focal length, divergencies ranging from 0.3 to 9.5 degrees are obtained. These correspond to spot sizes on the rhesus retina of $70 \mu\text{m}$ to 2.2 mm in diameter when the whole beam

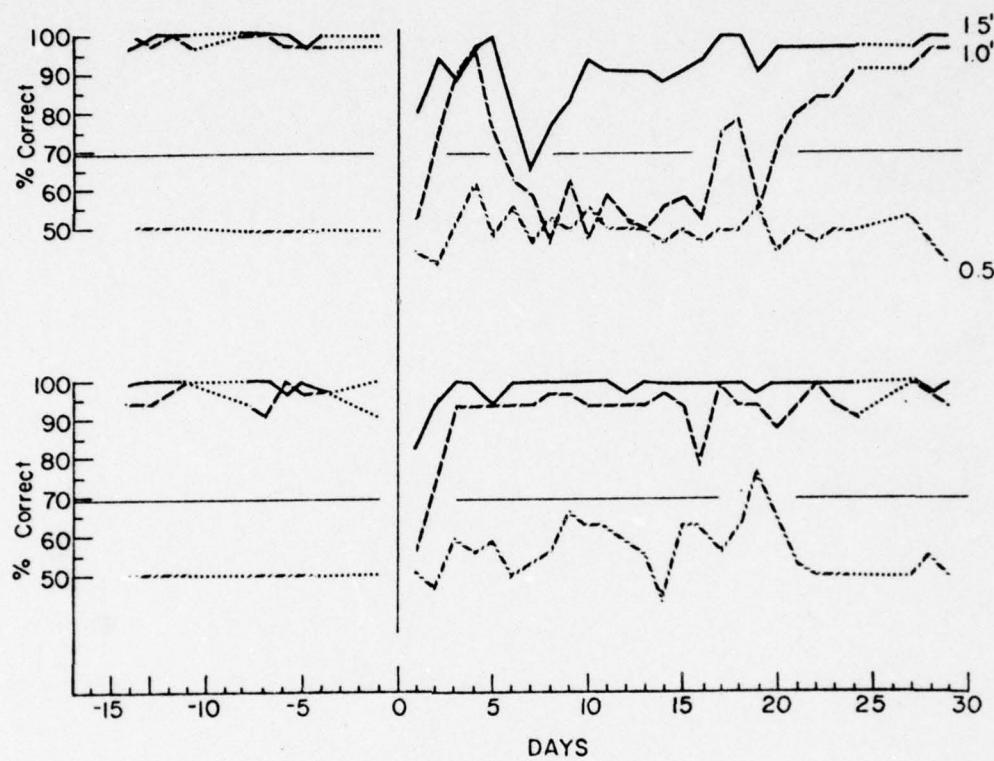


Figure 3

Twice Threshold (60 mW/cm² for 1000 s)

20/10 = 0.5'

20/20 = 1.0'

20/30 = 1.5'

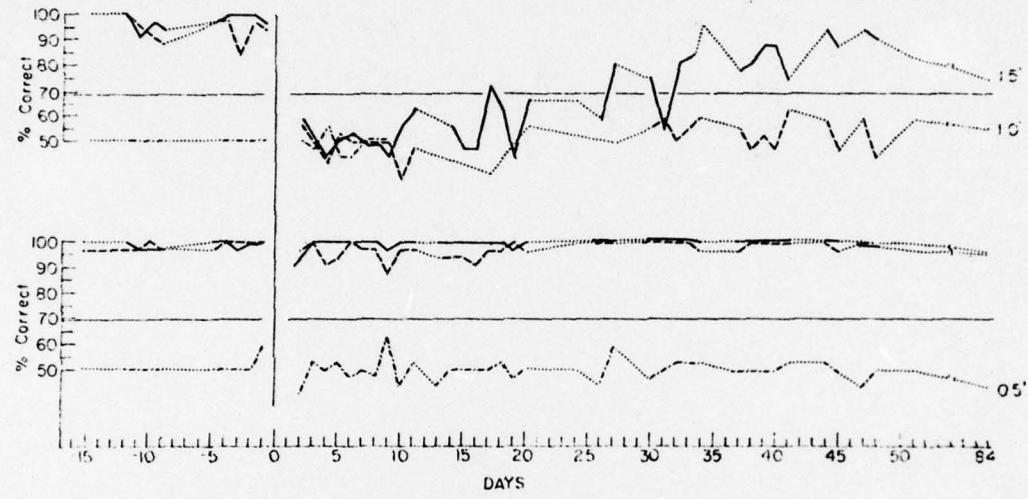


Figure 4

3 x Threshold (90 mW/cm² for 1000 s)

$$20/10 = 0.5'$$

$$20/20 = 1.0'$$

$$20/30 = 1.5'$$

SCHEMATIC DIAGRAM OF HIGH INTENSITY XENON SOURCE

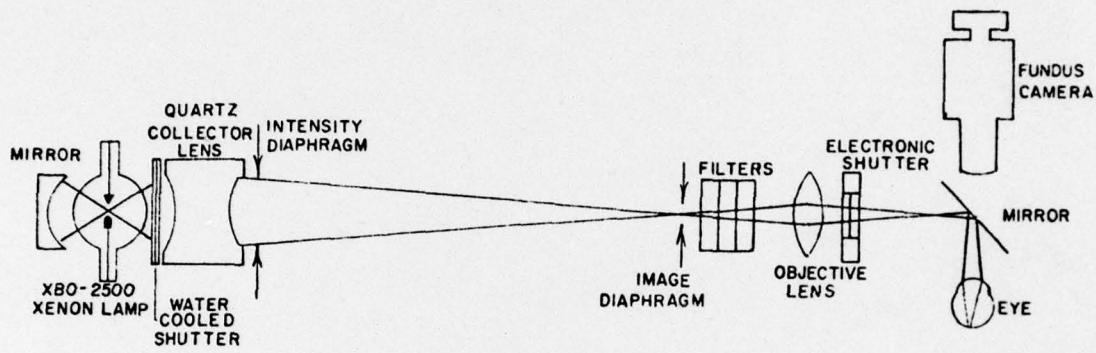


Figure 5

enters the eye. The irradiance on the retina can be varied by using stepped diaphragms in 5% increments, while preserving the spectral integrity of the source. The servo-controlled power supply maintains constant current to the lamp to within 0.1 A. Maximum current is 95 A. A Zeiss fundus camera is mounted in a fixed position, coaxial with the beam so that the cross-hairs of the camera focussed on the retina are coincident with the center of the radiation beam.

This source is multifaceted in its uses from 300-1400 nm. A "hot" mirror placed in the beam produces 2.1 W of power with a 9.5° divergence and a spectrum extending from 400-800 nm as shown in Figure 6. A "cold" mirror plus a 2-58 filter produces the infrared spectrum shown also in Figure 6. Approximately 1.8 W of infrared power are available. Current through the lamp is 86 A in both cases.

Another extremely useful spectral distribution in the near UV is obtained by a combination of 4 filters. Two spectral distributions are shown in figure 7. Approximately 48 mW are available between 330-420 nm with a lamp current of 86 A and a beam divergence of 9.5°, 120 mW are available for the 330-490 nm spectrum under similar conditions. These two spectra will be used to determine the effects of near UV radiation on the cornea, lens and retina, as described in the research protocol.

Still other combinations are available with this source. For example, the solar spectrum at sea level can be simulated fairly well from 300-1400 nm by using an Aerospace Control Corporation ER-7187 reflectance filter as shown in Figure 8. The power available is 4 W for a lamp current of 86 A and a beam divergence of 9.5°. By using narrow bandpass interference filters almost any part of the visible, infrared, or near UV spectrum is available at near monochromatic coherency. For example, such filters at 405 and 435 nm produce better than 4 mW of power. This is much better than the old xenon source which only allowed us to produce a threshold lesion in 1000 s. We are now in a position to get data at 16 and 100 s to fit in with our action spectrum at short wavelengths in the visible and near UV spectrum.

Another advantage of this source is the uniformity of irradiance perpendicular to the beam. Except for very small divergences there is very little rounding off near the edges of the image. This is, of course, in sharp contrast to a gas laser operated in the TEM_{00} mode which produces a Gaussian distribution across the beam. It is for this reason that we produced most of our "blue" light lesions for histological study with xenon source rather than with the 441.6 nm He-Cd laser. Also, the latter was defective and had to be sent back to the factory for repairs.

4. Threshold Data on ms Pulses of 441.6 nm Light

Preliminary data indicate that short pulses of 441.6 nm light are more effective in producing retinal lesions than an equivalent radiant exposure to CW 441.6 nm light. Using the He-Cd laser and an electronically controlled mechanical shutter we have exposed the rhesus retina to 1000 pulses of 441.6 nm light, 8 ms pulse width and 22 μ J/pulse. The spot size on the retina was 500 μ m in diameter to the $1/e^2$ points, corresponding to our previous data with CW exposures (Nature, 260,

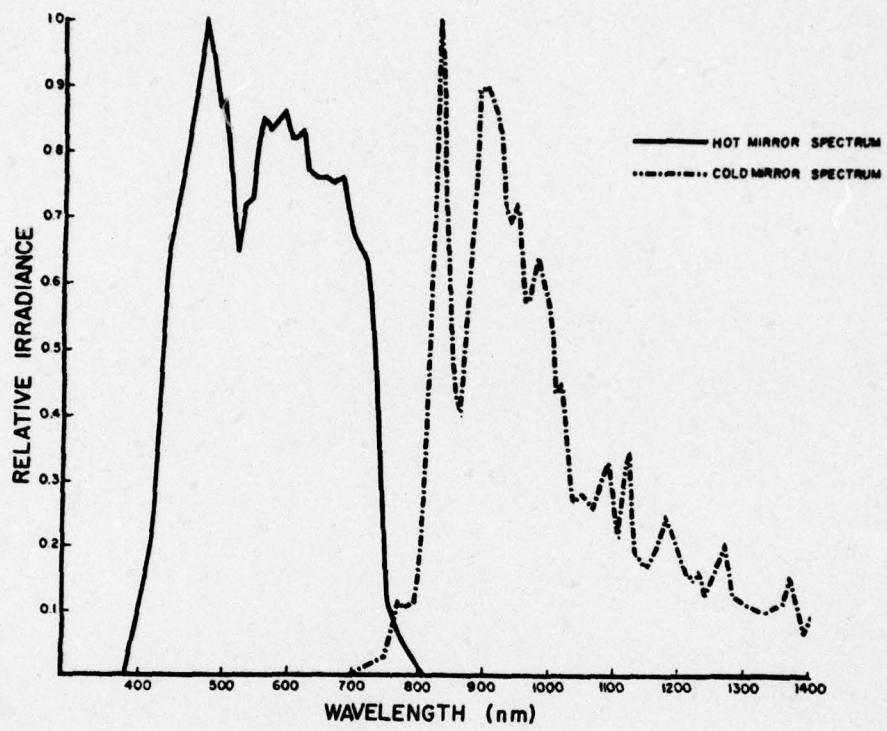


Figure 6

Continuous spectra produced by "hot" and "cold"
mirrors with xenon source

TWO SPECTRAL SOURCES PRODUCED WITH XENON
ARC AND MULTIPLE FILTER COMBINATIONS

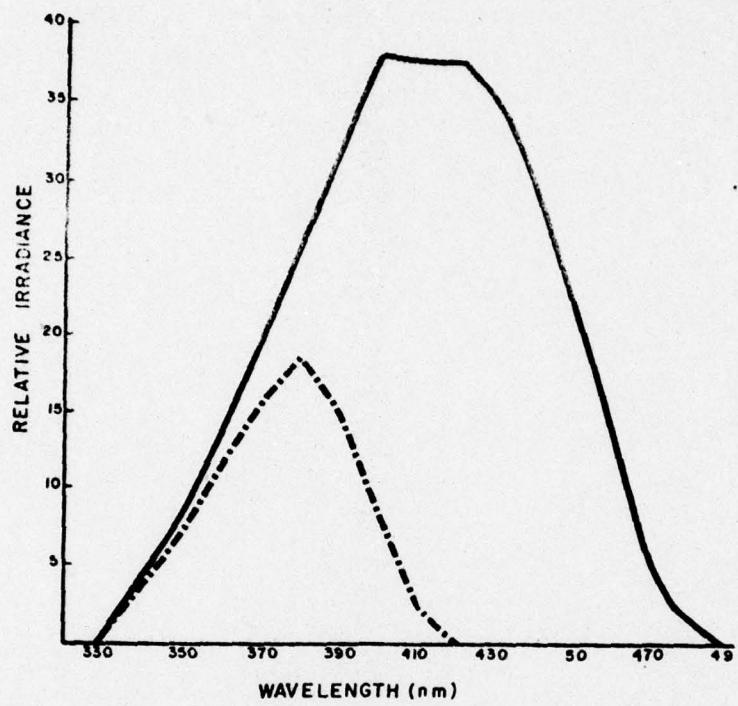


Figure 7

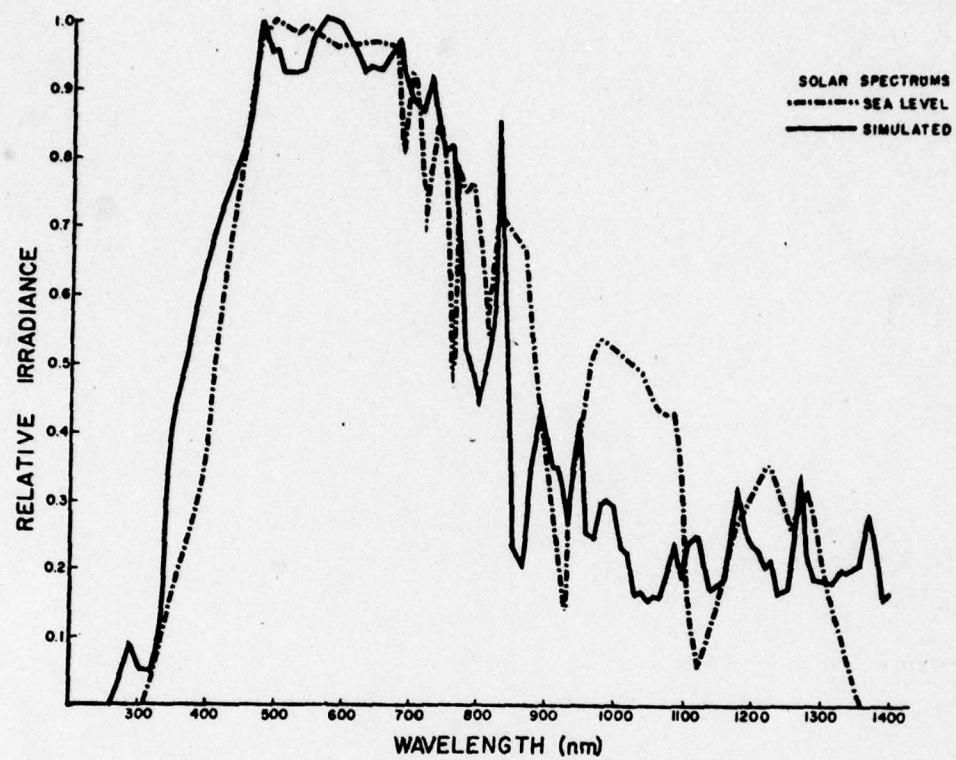


Figure 8

Solar spectral simulation produced with the xenon quartz optics source in conjunction with Aerospace Control Corp. ER 7187 reflectance filter. The simulated spectrum is compared with the solar spectrum at sea level.

153-155, 1976). A funduscopically visible lesion appeared 24 hours postexposure. This calculates out to be approximately a radiant exposure of 20 J/cm^2 as compared to 30 J/cm^2 for a 1000 s exposure to CW 441.6 nm light as produced by the He-Cd laser. The maximum temperature rise for a single 8 ms pulse is approximately 0.5°C , so that it is difficult to understand how temperature could play a part in this phenomenon. Presumably the interval between pulses (0.992 ms) is sufficient to insure return to ambient temperature before the next pulse arrives. This, however, requires checking by computations utilizing the more sophisticated models for heat conduction in the retina which are now available.

Another set of 8 ms pulses of $11.5 \mu\text{J}/\text{pulse}$ produced a minimal, very small lesion which was funduscopically visible 48 hours postexposure. The radiant exposure is calculated to be 10.5 J/cm^2 which is almost a factor of three below the CW threshold of 30 J/cm^2 for CW 441.6 nm light. These preliminary data are somewhat disturbing and seem to indicate that pulses of short wavelength light are considerably more damaging than CW exposure. Two possible explanations are: (1) There may be a small but definitive rise in temperature for succeeding pulses resulting in some thermal enhancement of photochemical effects, or (2) the photochemical processes leading to the lesion depend upon the rate of energy delivery or power density. The latter hypothesis seems the more likely and we hope to explore this phenomenon further as a function of power density per pulse and wavelength.

5. Preliminary Data on Near UV Effects on the Retina of the Aphakic Rhesus Eye

To date, three rhesus monkeys have undergone lens extraction in one eye (cryoextraction) by Dr. DuPont Guerry. Also, we received one animal whose lens had been extracted in one eye from Dr. Harry Zwick of LAIR. Unfortunately, this animal died several months after arrival in our laboratory from cause unknown but not connected with the lens extraction operation. We are confident from past experience that lens extraction in the rhesus monkey presents no major problems.

To date, we have exposed one aphakic eye to the 351 and 364 nm lines of the argon laser. The unfocussed beam produces a spot size on the retina of approximately $500 \mu\text{m}$ to the $1/e^2$ points when the lens is missing. We succeeded in making 6 exposures of 100 s duration and 4 exposures of 16 s duration before our argon laser became inoperative due to a leak in the tube seal. The entire tube has to be replaced before we can resume 351 and 364 nm exposures. Our results to date are shown in the following table:

Exposure	Corneal Power in W	Retinal Irrad. W/cm^2	Rad. Exp. J/cm^2	Exp. Time s	Remarks
1	.00497	2.53	253.2	100	lesion
2	.00133	0.678	67.7	100	lesion
3	.000092	0.047	4.7	100	negative
4	.00117	0.595	59.5	100	lesion
5	.00086	0.436	43.6	100	lesion
6	.000607	0.309	30.9	100	lesion
7	.00529	2.69	43.1	16	lesion
8	.0047	2.39	38.3	16	lesion
9	.0036	1.83	29.3	16	lesion
10	.0032	1.63	26.0	16	lesion

These calculations are based on a transmittance through cornea, aqueous and vitreous of 0.5 as calculated from the data of Boettner and Wolter (Invest. Ophthal. 1, 776, 1962). The appearance of the lesions is shown in Figure 9, which is a fundus photograph in which each exposure is numbered on the film. Obviously exposure 1 is severe and far above threshold. Exposure 3 was a mistake (wrong filter), but we include it to show that no lesion appeared. Exposure 6 (30.9 J/cm^2) was the last one we made before the laser broke down. Judging by its appearance in Figure 9 we would say it is well above threshold. A radiant exposure of 20 J/cm^2 was threshold for the 441.6 nm blue line from the He:Cd laser for a 100 s exposure. For the 16 s exposures threshold was never reached. The 441.6 nm 16 s exposure threshold was 6.6 J/cm^2 . Judging by these preliminary results the retina is extremely sensitive to near UV damage, but we have not yet shown that it is as sensitive to the near UV as to the blue light (441.6 nm). We intend to obtain threshold data for 1, 16, 100 and 1000 s at several points in the near UV using the He:Cd 325 nm line, the two argon lines 351 and 364 separately, and two or more other portions of the near UV spectrum by using the xenon lamp source with narrow bandpass interference filters. We also intend to get a 1000 s threshold for the UV spectra shown in Figure 7, namely 330-420 nm and 330-490 nm. It will be necessary to use aphakic eyes for this program, but by using small spot sizes on the retina ($500 \mu\text{m}$ diameter) we hope to obtain a maximum amount of data from each eye.

We have exposed one normal eye (lens intact) to 6.44 mW of 351 and 364 nm unfocussed argon radiation for 1000 s. The beam diameter at the cornea was 2.3 mm, so that the corneal peak irradiance was 310 mW/cm^2 and the peak radiant exposure 310 J/cm^2 . This produced a severe corneal lesion but this cleared up completely by the fourth day postexposure so that we were able to detect a small retinal lesion approximately $50 \mu\text{m}$ in diameter. If one assumes a transmittance through the ocular media of 0.005, calculation gives a peak retinal irradiance of 3.3 W/cm^2 and a peak radiant exposure of 330 J/cm^2 . Since this is well above threshold for the retina as shown above we can only conclude that the transmittance through the ocular media when the lens is present must be at least a factor of ten less than .005. This would reduce the radiant exposure to about 33 J/cm^2 which is more in keeping with the observed appearance of the tiny white lesion $50 \mu\text{m}$ in diameter. Two other factors which may provide additional protection to the retina are the loss of dioptic power at these short wavelengths which tends to spread the retinal spot and Rayleigh inverse 4th power scattering which insures a large amount of scattering at the shorter wavelengths.

6. Repetitive Subthreshold Exposures of Short Wavelength Light (441.6 nm) to the Rhesus Retina

Having demonstrated the extreme sensitivity of the mammalian retina to short wavelength light, a natural sequel is to investigate as to whether subthreshold exposures are cumulative. We have made some limited attempts to answer this question by exposing the same area on the retina to repetitive subthreshold radiant exposures.

The xenon lamp source with narrow bandpass filter at 441.6 nm was used to expose the retina for 1000 s, using a 4.5° beam divergence which produces a spot size on the rhesus retina approximately 1 mm in diameter. Since the threshold for 441.6 nm is 30 mW/cm^2 on the retina for 1000 s, we chose one-half threshold, or



Figure 9

Fundus photograph of lesions produced on the retina
by the combined 351 and 364 argon lines in the near
UV (See table in text).

15 mW/cm² and exposed the same paramacular area for 1000 s on two occasions separated by 48 hours. This did produce a lesion. We then reduced the retinal irradiance to 1/4 or 7.5 mW/cm² and exposed the same paramacular area four times with an interval of 48 hours between exposures. No lesion was funduscopically visible. We continued to exposre this same area 10 times, with 48 hours between exposures. There was no fundusscopic evidence of a lesion.

Obviously these two sets of experiments are only a beginning, and we should like to interpolate between them, but this is a time consuming and difficult task. Now that we have monkeys trained for a visual task it seems more appropriate to use them to investigate the effects of cumulative subthreshold exposure on visual function. We have already shown that one paramacular threshold exposure of the macula produces no loss of visual function. Will this be true for repetitive exposures of the macula at 24, 48, or 72 hours between exposures? Another approach involves using the techniques of light and electron microscopy to detect damage from repetitive exposures which are funduscopically visible. We intend to search for damage at levels below the funduscopically visible threshold, using histological techniques and ultrastuructural investigation.

7. Extension of Action Spectrum to Shorter Wavelengths in Visible and Near UV Spectrum

Attempts to get reliable threshold data at 435 and 405 nm and in the near UV have been stymied by a number of obstacles. Obviously, the only source of near UV data is on the aphakic eye and this research is now well in progress as outlined in section 5. Transmittance through the ocular media begins to drop drastically below 441.6 nm, reaching about one percent at 405 nm. Our major difficulty is the uncertainty or large standard deviation in the transmittance at 435 and 405 nm. This is well illustrated in Figure 10 which shows the standard deviations for transmittance as measured on 9 eyes.

Using the old xenon lamp with a narrow bandpass filter at 435 nm we obtained a threshold lesion at 20 mW/cm² on the retina for 1000 s. This source was not powerful enough to produce lesions at 100 or 16 s. The threshold value is uncertain to \pm 5 mW/cm² but seems to indicate that the action spectrum for a threshold lesion is still advancing at 435 nm. Knowing that melanin absorbs strongly in the UV and taking into consideration what we now know about the role of the melanosomes in the photochemical lesion as outlined in Section 1 of this research report, there is every reason to believe that the retina is extremely sensitive to UV radiation. The preliminary data obtained so far on the aphakic eye as outlined in Section 5 support this belief.

Obviously, at 405 nm we did not have enough power to produce a retinal lesion with the old xenon source, even for 1000 s exposure durations. Using the new source described in section 3, we can produce better than 4 mW through narrow bandpass filters at 435 and 405 nm. We should therefore be able to complete the action spectrum for those two wavelengths in the near future.

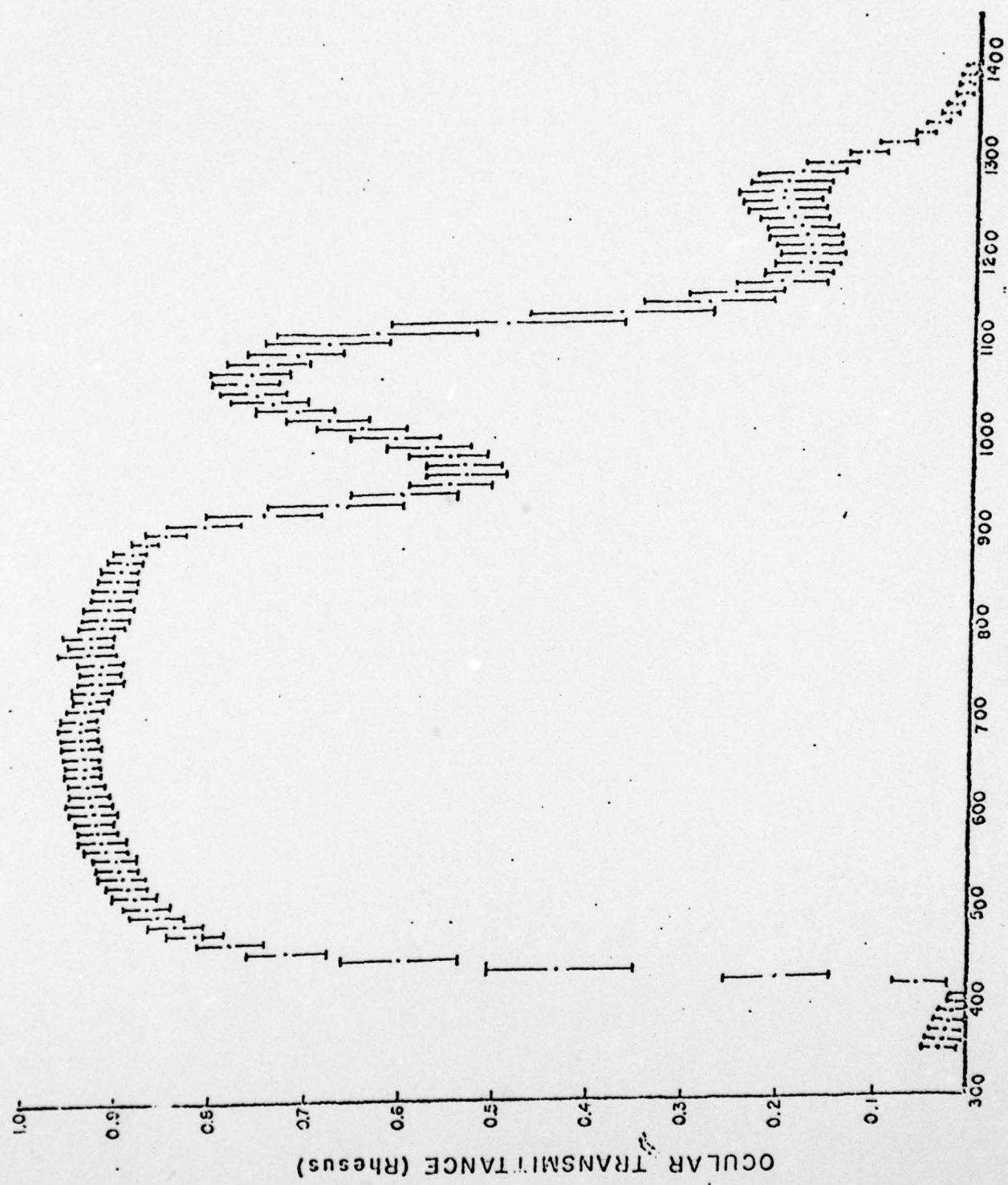


Figure 10 Transmittance through the ocular media of the rhesus monkey as measured on 9 eyes from 5 animals.

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20. Preliminary data on 1000 eight ms pulses of 441 nm light at a one pulse per second repetition rate indicate that the radiant exposure required to produce a threshold lesion is lower than for cw exposure. Some preliminary exposures of the retina of aphakic monkeys support the thesis that the retina is quite sensitive to near ultraviolet damage. Also, repetitive exposures to blue light show that cumulative damage can occur in the retina.

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